PB-237 908-T

ON THE QUESTION OF THE MODE OF ACTION OF THE BRONCHOSECRETOLYTIC AGENT, N-CYCLOHEXYL-N-METHYL-(2-AMINO-3, 5-DIBROMOBENZYL)-AMMONIUM CHLORIDE

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National Institutes of Health

Prepared for:

Environmental Protection Agency

1974

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PB 237 908-T
EPA-TR-84-74

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NIH-74-05C

Arzneim-Forsch
17:353-355, 1967

ON THE QUESTION OF THE MODE OF ACTION OF THE BRONCHOSECRETOLYTIC AGENT, N-CYCLOHEXYL-N-METHYL-(2-AMINO-3, 5-DIBROMOBENZYL)-AMMONIUM CHLORIDE

By

Von H. Eigelsreiter and M. Mair

Although it appears to be certain that N-Cyclohexyl-N-methyl-(2-amino-3, 5-dibromobenzy)-ammonium chloride (Bisolvon®) has an expectoration-promoting effect in animals, (1, 2) and human beings (3, 4), the mechanism of action has not been thoroughly elucidated. Beside the reduction of viscosity of the bronchial secretion (5, 6) in the sense of a secretolysis, for which Merker (7) could adduce, with the help of an electron microscope, a considerable increase in the granula of the secretion and of lysosomes in the caliciform cells, there also exists a dispute over an increased absorption from the bronchial tract (8), which Gordonoff (9) also views as a function of the secretolytic agents. In the present examination an experiment was undertaken for the purposes of further clarification of the mechanism of action of Bisolvon.

Method

The experiments were carried out on the isolated guinea pig lungs according to the method of Delaunois (10, 11).

The lung, which was fixed in the condition of inhaling by means of closing off the trachea, was placed in an artificial plexiglas thorax, where (after removal of the tracheal valve) it is caused, with the help of a pump that creates a vacuum in the artificial thorax, to "breathe". The underpressure could be read off a manometer. In our experiments, the underpressure was established generally at around 29 mm Hg. The necessary ventilating air , which is inhaled upon expansion of the lung, was removed pressurelessly from a compressed-air current flowing by continuously. The breath expulsions were transmitted to a cymograph with the help of a Marey capsule. Reversal to a gauged spirometer allowed for the registering of the breath volume.

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Through the Arteria pulmonaris, the isolated lung was supplied continuously with fluid*). This fluid drops freely from the lung and collects on the floor of the artifical thorax. The velocity of its flow through the lung was established at such a speed that a drop fell usually after every third breath. Pharmaca could be added to the fluid by means of a spray attachment on the cover of the artifical thorax. A built-in heating unit with a thermostat and ventilator kept the temperature in the thorax space steadily at 38°C. Two aerosolators in the compressed-air current** allow for the admixture of various pharmaca in the form of aerosols to the "breathing-air current". In our experiments, acetylcholine and histamine in aerosol form were used as constricting substances, and adrenalin was used as a dilating pharmacon. Bisolvon was used as an aerosol at a dilution of 1:10 to 1:20 of the original solution (1 ml = 2 mg Bisolvon). Stronger dilutions revealed no effects. Higher concentrations led to edema.

Results

In a series of experiments on 5 isolated lungs it was shown that Bisolvon itself, in the concentration and dosage which later proved to be effective, has neither a constrictive nor a dilatory effect. (Fig. 1). For this reason, further experiments were carried out only in combination with constrictory or dilatory substances.

1. Acetylcholine

The known bronchoconstrictive effect of acetylcholine (12, 13, 14, 15) was also revealed in our experiments on 5 isolated lungs, with results that were sometimes alike. Acetylcholine given in 5 (4-6) breaths as an aerosol (2%), caused a constriction practically immediately. If additionally Bisolvon (0.01%) was introduced as an aerosol in 3 (2-4) breaths at intervals of 1-2 minutes, edema appeared within 4-8 minutes (Fig. 2). If the lung is handled precedingly with Bisolvon (same dosage as above), the effect of acetylcholine is not in any case prevented. Edema still appears. In both cases, no more perfusion fluid drops off (Fig. 3).

2. Histamine

The bronchoconstrictive effect of histamine is a fact which has likewise long been known (16, 17, 18, 19). The constriction caused by histamine can be lifted by means of sympathicomimetic agents (20, 21). If in such a case the sympathicomimetic agent is applied in a sufficiently high dose, then even the later given dose of constrictive pharmaca remains without effect [20]. In order to uncover a possible histamine-antagonistic effect of Bisolvon, we proceeded in our experiments with 33 isolated lungs in the following manner:

*See page 5
** (0.5 kg/cm^2)

After 2-5 breaths of a histamine-aerosol (5% solution), the expected constriction appeared. Now an average of 4 (2-8) breaths of Bisolvon were given. Immediately thereafter the constriction was brought to a stop in 29 of the total 33 isolated lungs, i.e. the breath expulsions did not become any smaller. In 17 cases, further Bisolvon doses (3-5 breaths at an interval of 1/2 to 1 1/2 minutes) caused a slight increase in the breath expulsions, whereby we were able to determine an increase in the breath volume (Figs. 4 and 5). This increase, even upon more frequently repeated Bisolvon inhalations, was no greater than after a few (4-8) breaths, an observation which had already been made earlier in the case of sympathicomimetic substances [22]. Thus, in the dose we had used, Bisolvon can prevent the appearance of a bronchial cramp produced in the isolated guinea pig lung by histamine.

If the isolated lung was pretreated with Bisolvon (3-6 breaths at an interval of 1/2 to 2 minutes, repeated 2-4 times), histamine inhalation leads nonetheless to constriction, which, however, can be halted by a new application of Bisolvon (dosage as above).

In all the experiments, fluid was flown through the lungs in such a manner that a drop of the perfusion fluid ran off the lung after every 3 breath expulsions. After inhalation of histamine, a drop would fall off only after every 6-8 breath expulsions; thus fluid was kept back in the lung. Addition of Bisolvon speeded up the drops to 1 drop in 4 breaths, and gradually the original drop count was re-established.

On the basis of the above findings, we suspected an increased perviousness of the vaso-bronchial barrier under the influence of Bisolvon. For this reason, we repeated the experiments with simultaneous adrenalin doses (5 γ/ml perfusion fluid). In 3 experiments, lungs which had not been treated preparatorily were brought to constriction by means of five breaths of histamine, and then converted to the adrenalin-containing perfusion solution. The breath expulsions became even smaller, then after 3, 4, 5 or 6 minutes the adrenalin effect set in and the breath exchanges became greater again. The maximum was reached after 6 - 6.5 minutes. In 3 experiments, the lungs were first pretreated with adrenalin-containing solution. After 5 breaths of histamine, the breath exchanges decreased only during 1.5, 1.25 or 1.0 minutes. The increase of the breath exchanges which then set in, under the influence of the pre-treatment with adrenalin, reached its maximum after 4 - 6.5 minutes.

In another series of experiments, on 10 isolated lungs, Bisolvon was introduced after the constriction effect through histamine inhalation (4-8 breaths) simultaneously with the change to adrenalin-containing perfusion fluid; the Bisolvon was introduced in 2-4 breaths. In all the cases, the decrease of the breath

exchanges immediately ceased; the increase which then set in reached its maximum after 3-7 minutes. In the case of simultaneous Bisolvon inhalation, the adrenalin effect was not hindered, but set in immediately (Fig. 6).

In the case of 6 of these 10 experiments the breath volume could also be determined with the spirometer. Before the commencement of the experiment, the breath volume amounted on the average to 3.1 ml (2.47-3.96 ml), which corresponds to the values cited in the literature [23]. After histamine-inhalation, the breath volume sank on the average by 42% (16-67%). After the subsequent Bisolvon-inhalation, the breath volume reached on the average 103% (88-138%) of the initial value.

Discussion of the Results

Bisolvon inhalation, after preceding acetylcholine inhalation, leads to the retention of the perfusion fluid in the isolate guinea pig lung and to a state of edema. The retention of perfusion fluid following histamine inhalation is lifted by Bisolvon inhalation. The constriction appearing upon acetylcholine inhalation is not prevented by Bisolvon inhalation. On the other hand, the constriction caused by histamine inhalation is halted by Bisolvon inhalation. The setting in of the effect of adrenalin (in the perfusion fluid) after previous constriction due to histamine inhalation is speeded up by Bisolvon inhalation.

We should like to express these findings in the following manner:

Under the effect of Bisolvon there is probably the rise of greater perviousness of the vaso-bronchial or alveolar barrier, which is noted upon the appearance of fluid in the alveoles or bronchi. The effect of Bisolvon on the histamine constriction can only be explained as an antihistamine effect. This, however, is contrasted by the fact that various antihistaminic agents can themselves have a constrictory effect [24]. It might, therefore, be also a matter of a "washing out effect" (rapid deportation of histamine). The fact of the easy conquerability of the vaso-bronchial barrier is supported also by the rapid appearance of effect of adrenalin in the case of simultaneous dosage of Bisolvon and the increased secretion.

Summary

The effect of N-Cyclohexyl-N-methyl-(2-amino-3.5-dibromo-benzyl)-ammonium chloride (Bisolvon) on the bronchial constriction caused by acetylcholine or histamine was studied in 54 isolated guinea pig lungs. The findings lead one to suspect - besides a possible antihistamine effect - an increased perviousness of

the vaso-bronchial barrier with increased secretion under the influence of Bisolvon.

*Perfusion fluid: 9.0 NaCl; 0.24 $CaCl_2$; 0.42 KCl; 0.3 $NaHCO_3$; 0.214 $MgCl_2 \cdot 6\ H_2O$; 1.0 glucose; aqua bidest. ad 1000; adjusted to pH 7.4 with NaH_2PO_4 (0.25%).

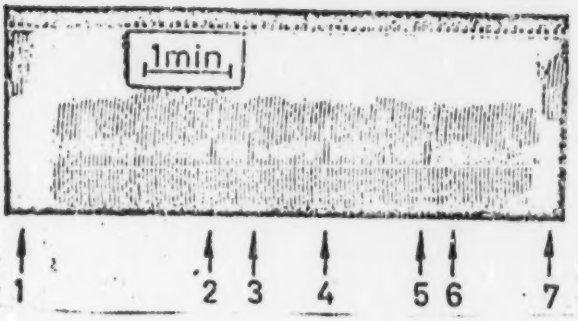

Fig. 1. The effect of 5x2 breaths of Bisolvon (0.01%) on lung expansion. Guinea pig no. 4, 568 g body weight. 4 lung lobes open freely and evenly. Breath canal open on index 150. Flow-through: 1 drop of perfusion fluid every 3 breaths. 1=breath volume 10.6 ml (spirometer measurement). 2,3,4,5, 6=every 2 breaths of Bisolvon. 7=breath volume 10.6 ml (spirometer measurement) no alteration in the lung expansion.

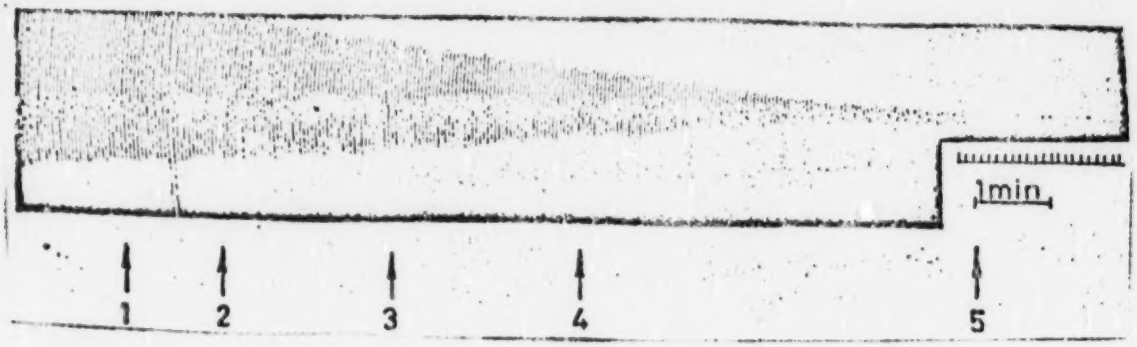

Fig. 2. The effect of 2x2 breaths of acetylcholine (2%) on the lung expansion. Guinea pig no. 9, 430 g body weight. 4 lung lobes open freely and evenly. Flowthrough: 1 drop every 3 breaths. 1,2 = every 2 breaths of acetylcholine constriction. 3,4 = every 4 breaths of Bisolvon (0.01%) constriction continues. 5 = edema.

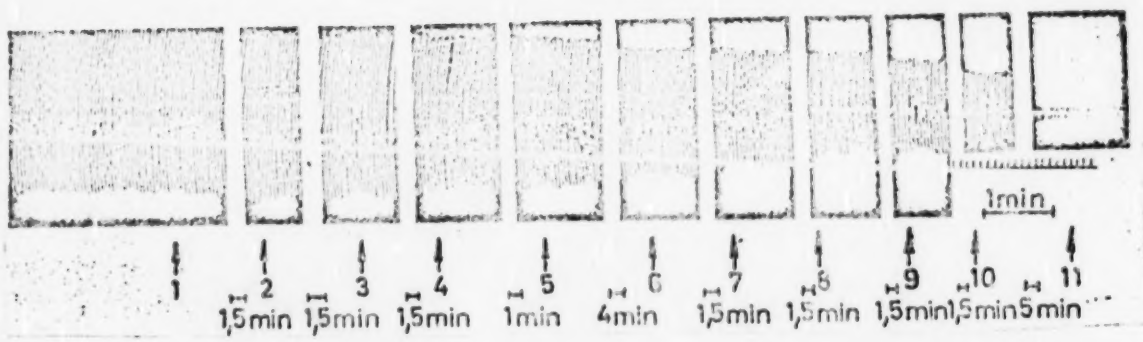

Fig. 3. The effect of 3x2 breaths of Bisolvon (0.02%) + 2x1 breaths acetylcholine (2%) on the lung expansion. Guinea pig no. 10, 554 g body weight. 4 lung lobes open freely and evenly. Flowthrough: 1 drop every 3 breaths. 1,2, and 3 = every 2 breaths Bisolvon (0.02%) no change. 4 and 5 = every breath acetylcholine (2%) constriction. 6 to 10 = every 2 breaths Bisolvon constriction continues. 11 = edema.

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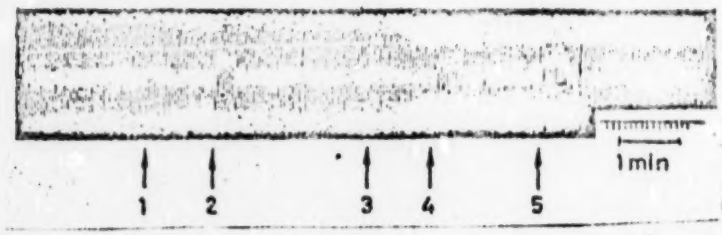

Fig. 4. Effect of 3 or 4 breaths of histamine (5%) + 6 or 16 breaths Bisolvon (0.01%) on the lung expansion. Guinea pig no. 19, 465 g body weight. 4 lung lobes open freely and evenly. Flowthrough: 1 drop every 3 breaths. 1=3 breaths of histamine lead to constriction. 2=6 breaths Bisolvon lead to stabilization (constriction does not continue). 3=4 breaths histamine lead to constriction. 4=8 breats Bisolvon lead to stabilization, 5=8 Bisolyon (no further change).

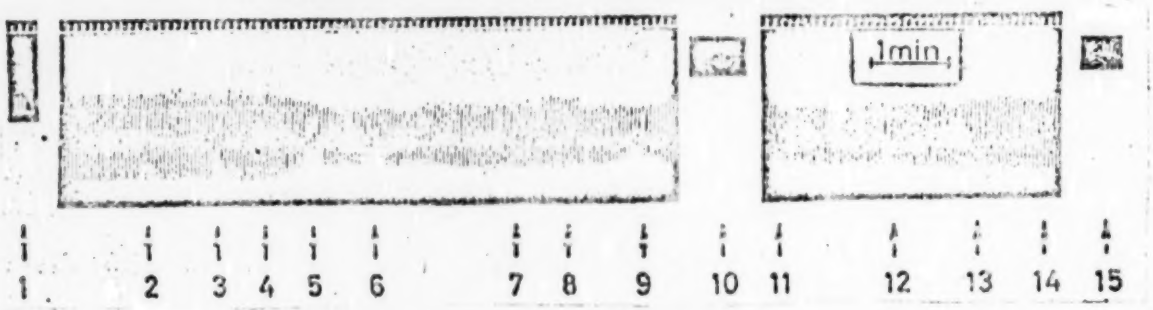

Fig. 5. Effect of 2x1 breaths histamine (5%) + 30 breaths Bisolvon (0.01%) on the lung expansion. Guinea pig no. 44, 437 g body weight. 4 lung lobes open freely and evenly. Flowthrough: 1 drop every 3 breaths. 1=breath volume 1.4 ml (spirometer measurement). 2 and 3 = every 3 breaths Bisolvon. 4 - 1 breath histamine leads to constriction. 5,6 and 7 = every 3 breaths Bisolvon lead to stabilization (constriction does not continue growing). 8 = 1 breath histamine leads to constriction. 9 = 3 breaths Bisolvon lead to stabilization. 10 = breath volume 0.885 ml (spirometer measurement). 11 to 14 = every 3 breaths Bisolvon lead to dilatation. 15 = breath volume 1.035 ml (spirometer measurement).

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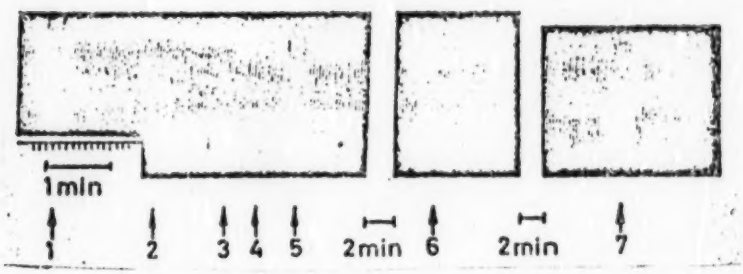

Fig. 6. Effect of 2x2 breaths histamine (5%) + 3 breaths Bisolvon (0.02%) on the lung expansion. Guinea pig no. 24, 588 g body weight. 4 lung lobes open freely and evenly. Flow-through: 1 drop every 3 breaths. 1 = breath volume 3.45 ml (spirometer measurement). 2 and 3 = every 2 breaths histamine lead to constriction. 4 = 5 µg adrenalin per 5 ml perfusion fluid leads to stabilization after 4 minutes. 5 = breath volume 2.47 ml (spirometer measurement). 6 = 3 breaths Bisolvon lead to dilatation. 7 = breath volume 3.47 ml (spirometer measurement).

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